Genetic Mapping of Soluble Guanylyl Cyclase Genes
Implications for Linkage to Blood Pressure in the Dahl Rat

Mohammad Azam, Gopa Gupta, Wei Chen, Sandra Wellington, Dorothy Warburton, Robert S. Danziger

Abstract—The nitric oxide (NO) signaling system, consisting of NO synthases, soluble guanylyl cyclase, and cGMP, plays a prominent role in salt handling and regulation of blood pressure. Soluble guanylyl cyclases are heme-containing heterodimers (α/β). The α1/β1 isofrom has greater NO sensitivity than the α1/β2. It has recently been shown that expression of the β subunits is altered in the kidney of the Dahl salt-sensitive rat, ie, the β1 subunit is decreased and the β2 subunit increased. However, whether soluble guanylyl cyclase is linked to salt sensitivity is not known. In the present study, we investigated linkage of guanylyl cyclase genes to blood pressure. α1 and β1 gene loci for soluble guanylyl cyclase were mapped to rat chromosome 2, and the β2 gene locus was mapped to rat chromosome 5 using fluorescent in situ metaphase hybridization. By use of a rat radiation hybrid panel, the gene loci were then further mapped with respect to known quantitative trait locus markers of salt-sensitive hypertension in the Dahl rat on chromosomes 2 and 5. Genes for α1 and β1 were closely linked by two-point analysis to Na+,K+-ATPase α1 isofrom (LOD of 15.1 and 14.0, respectively) and calmodulin-dependent protein kinase II-δ loci (LOD of 14.3 and 12.9, respectively), which have been previously shown to flank a quantitative trait locus for blood pressure in the Dahl rat. The α1 and β1 genes were closely linked (LOD of 11.3; δ, 0.4). The β2 gene locus was closely linked to the endothelin-2 (ET-2) locus (LOD of 13.0), which has been shown to cosegregate with blood pressure. We conclude that soluble guanylyl cyclase subunit loci, ie, α1, β1, and β2, are good candidates for genes controlling salt-sensitive hypertension in the Dahl rat. (Hypertension. 1998;32:149-154.)

Key Words: guanylyl cyclase ■ cyclic GMP ■ genetics ■ hypertension, salt-sensitive

The NO signaling system, consisting of NO synthases, SGC, and cGMP, plays a prominent role in salt handling and regulation of blood pressure. In biological systems, SGCs (GTP pyrophosphate-lyase [cyclizing; EC 3.6.1.2]) are preeminent receptors for NO. NO and carbon monoxide activate guanylyl cyclase enzymes by complex formation with an associated heme moiety, which forms the catalytic domain and shares significant homology with the carboxyl terminus of membrane-bound guanylate cyclases and adenylate cyclases. The amino termini are less homologous and are important in the formation of the catalytic domain and shares significant homology with the carboxyl terminus of membrane-bound guanylate cyclases and adenylate cyclases. The amino termini are less homologous and are important in the binding and/or signal transduction of the stimulatory binding signal, ie, NO, to the catalytic domain. SGC was first purified from the bovine lung, from which the α1 and β1 subunit isofroms were first cloned. Human* and rat forms of α1 and β1 have subsequently been identified and cloned. Message for the α1 and β1 subunits has been detected by Northern blot analysis in lung, cerebellum, kidney, heart, liver, skeletal muscle, olfactory mucosa, and tongue. Thus, the α1/β1 isofrom is the universal form. Another isofrom of the β subunit, β2, has been identified by homology screening and was cloned from a rat kidney library. Northern blot analysis indicates that it is principally expressed in the kidney and liver. We have previously shown that the α1 and β2 subunits can form a catalytically active, NO-stimulated heterodimer that has lower specific activity than the α1/β1 heterodimer. On this basis, we postulated that increased expression of the β2 subunit can decrease NO-stimulated guanylyl cyclase activity in cells.

The SS/Jr is a widely studied model of genetic hypertension. Kidney cross-transplant studies between SS/Jr and SR/Jr have demonstrated that the genotype of the kidney plays a significant role in the pathogenesis of salt-induced hypertension in the Dahl rat. *Although originally reported as α3 and β3, it is generally accepted that these are human forms of α1 and β1 due to sequence homology.
hypertension. However, the difference between the SS/Jr and SR/Jr kidney at a molecular level remains unclear. We have recently reported that expression of the β2 guanylyl cyclase subunit is increased and that of the β1 subunit is decreased in the kidney of the SS/Jr compared with the SR/Jr. Whether guanylyl cyclase isoforms are genetically linked to blood pressure and salt sensitivity in the Dahl rat is unknown.

QTLs for salt-sensitive blood pressure in the Dahl salt-sensitive rat and gene loci cosegregating with blood pressure have been previously identified using crosses of SS/Jr with SR/Jr, WKY, and MNS rats. Loci segregating with blood pressure were found in chromosome 2, mapping at the GC-A and between the NAK and CAMK, and in chromosome 5 closely linked to the ET-2 locus. In the present study, we tested whether the genes for SGC map to chromosome regions previously identified as containing QTLs for salt-sensitive blood pressure to determine whether they are candidate genes for hypertension in the Dahl rat.

Methods

Chromosomal Localizations

FISH was performed on chromosomal spreads from rat EIC18 cells. Full-length rat cDNAs subcloned into pcDNA-neo were used as probes for the β1 and α1 genes (obtained as a gift from M. Nakane, Abbott, Ill). The probe for the β2 gene was a 22-kb genomic clone obtained by screening a rat genomic library (kidney) in the λ Dash II vector (Stratagene) using a 320-bp cDNA fragment obtained from an ApaI digest of the full-length cDNA subcloned into Bluescript Vector (obtained from P. Yuen, Memphis, Tenn). The β2 probe was random prime-labeled (Random Prime Labeling kit, Amersham), and filters were screened under high stringency. Individual clones were identified after the tertiary screen. λ DNA isolated by
the Qiagen Isolation System at a concentration of 10 μg/μL was used for hybridizations. Probes were labeled by nick translation with digoxigenin and hybridized at a concentration of 10 μg/μL overnight. Slides were washed in 1× SSC at 50°C, detected using antidigoxigenin-labeled FITC, counterstained with DAPI, and examined using a Nikon Microphot microscope and Cytovision Image Analysis System (Applied Imaging). Hybridizations were repeated two times. Enhanced DAPI images were karyotyped, and the location of hybridization signals was noted in 20 metaphase spreads.

Radiation Hybrid Mapping
A rat-hamster hybrid panel created by Peter Goodfellow (Cambridge, UK) was obtained from Research Genetics, Inc (Huntsville, Ala). To create the panel, a rat cell line (donor RatFR) was exposed to 3000 rad of x-rays and then fused with nonirradiated thymidine-deficient hamster recipient cells (A23). The panel consists of 106 clones and has an average locus retention rate of 28%.

The presence or absence of each marker was determined using PCR. Each marker was tested separately (none were multiplexed). The PCR primer sets for α1, β1, and β2 subunits of guanylyl cyclase; CAMK; NAK; ET-2; guanylate cyclase-A/atrial natriuretic peptide receptor-A (GC-A); and D2N35 were designed from published sequence data from Genebank (Table 1). PCRs were carried out in a total volume of 50 μL with 0.35 ng of DNA template, 300 nmol/L of each PCR primer, 15 mmol/L MgCl2, 200 μmol/L dNTPs, and 2.6 U of Expand polymerase (Boehringer Mannheim). The PCR profile consisted of 30 cycles of 94°C for 30 seconds (denaturation), 50°C for 60 seconds (annealing), and 72°C for 2 minutes (extension), followed by an additional 10-minute final extension at 72°C. Optimal annealing temperature was determined for each set of primers on the basis of GC content. The PCR products were resolved on a 2% agarose gel and analyzed using the Bio-Rad gel documentation system.

The radiation hybrid mapping program RHmapper was used to analyze the data. Two-point analysis was performed for gene loci known to cosegregate with blood pressure and SGC genes on appropriate chromosomes. The order of the CAMK, NAK, GC-A, D2N35, and SGC loci was determined by the stepwise ordering strategy with a machine-generated candidate order. Distances were calculated using the “evaluate function” and are reported in cR3000, where 3000 rad indicates the dosage of x-rays used in the irradiation of the hybrids.

Results

Chromosomal Mapping
By FISH, probes for both α1 and β1 were localized on metaphase spreads to rat chromosome 2, band q31. β2 mapped to the most distal band of chromosome 5. Specific signal was identified on at least one chromatid in 20 of 20 pairs examined (Figure).

Radiation Hybrid Mapping
The presence or absence of each of the markers in 92 of 106 radiation hybrid clones was determined by PCR screening using primers as outlined in Table 1. Each of the markers was
detected in 17% to 24% of the hybrids. Retention frequency of the CAMK, NAK, ET-2, GC-A, and D2N35 loci ranged from 17% to 22%. The retention frequency for the \( \alpha_1 \) loci was 22% and for the \( \beta_2 \) loci 24%.

LOD score from two-point analysis (Table 2) showed close linkage of the \( \alpha_1 \) and \( \beta_1 \) gene loci to CAMK, D2N35, NAK, and GC-A with LOD of 9.5 to 15.1 on chromosome 2. The closest linkage determined by two-point analysis for both \( \beta_1 \) and \( \alpha_1 \) was with CAMK and NAK, which are loci previously shown to flank a QTL for blood pressure.\(^9\) Linkage between \( \alpha_1 \) and \( \beta_1 \) was also suggested because \( \theta \), defined as the probability that two loci are separated by one or more irradiation-induced breaks and an estimate of the physical distance between the markers, was 0.40 with an LOD of 11.3. The loci on chromosome 2 were subjected sequentially to analyses for order. For \( \beta_1 \) the most likely order was GC-A, NAK, ET-2, CAMK, D2N35, and for \( \alpha_1 \) it was GC-A, NAK, \( \alpha_1 \), CAMK, D2N35, with LOD versus next best of 0.5 and 0.4, respectively. The most likely order for the marker genes, ie, GC-A, NAK, CAMK, D2N35, corresponds to that determined using linkage analysis. Together, the data support a close linkage of \( \alpha_1 \) and \( \beta_1 \) gene loci between NAK and CAMK on rat chromosome 2.

The ET-2 locus was closely linked to the \( \beta_2 \) locus on chromosome 5, with a LOD of 13.0. The distance between the ET-2 and \( \beta_2 \) loci on chromosome 5 was calculated to be 53.6 cR\(_{\text{1000}}\). Although the resolution of the rat-hamster radiation hybrid panel has not been determined, based on the resolution of human and mouse radiation hybrid panels obtained with 3000 rad, a distance of 4 to 11 Mb is estimated.

**Discussion**

The present study suggests a link between SGC genes and salt sensitivity in the Dahl rat. We have shown that the genes for SGC \( \alpha_1 \) and \( \beta_1 \) subunits map to chromosome 2 and are closely linked to the GC-A locus, which has been shown to cosegregate with blood pressure,\(^{20,24}\) and the NAK and CAMK genes, which have previously been shown to flank a QTL for blood pressure in the Dahl salt-sensitive rat in F\(_2\) populations of male rats derived from crosses of Dahl salt-sensitive with WKY and MNS rats (LOD score of 5.66 based on the combined population).\(^{20,24}\) Our data also demonstrate that the \( \beta_2 \) and ET-2 gene loci on chromosome 5 are closely linked, indicating that the \( \beta_2 \) gene for guanylyl cyclase will also cosegregate with blood pressure, since the ET-2 locus has been shown to cosegregate strongly with systolic blood pressure in an F\(_2\) population derived from a cross between Dahl salt-sensitive and Lewis rats.\(^{21}\)

Pharmacological studies have demonstrated that renal NO signaling and cGMP regulate salt sensitivity and hypertension.\(^{20-35}\) Inhibitors of NO formation, such as \( \text{N}^{\text{G}} \)-monomethyl-L-arginine, reduce sodium excretion and increase both arterial blood pressure and salt-induced increases in arterial pressure.\(^{31,34,35}\) In addition to influencing sodium handling by regulating renal blood flow, there is growing evidence that NO affects renal sodium transport in the absence of hemodynamic effects, most likely through inhibition of \( \text{Na}^+ \text{-K}^+ \text{-ATPase} \),\(^{36}\) \( \text{Na}^+ \text{-H}^+ \) exchange in the proximal tubule,\(^{37}\) and sodium transport in cortical collecting duct cells.\(^{38}\) In the Dahl salt-sensitive rat, administration of \( \text{L} \)-arginine, a substrate for NO synthases, prevents the development of hypertension,\(^{39,40}\) normalizes pressure natriuresis,\(^{40}\) and increases the glomerular filtration rate.\(^{42}\) Recent studies have demonstrated that as in other tissues, the effect of NO in the kidney is mediated by cGMP generated by SGC. cGMP has been directly linked to inhibition of \( \text{Na}^+ \text{-H}^+ \) exchange in the proximal tubule,\(^{37}\) to regulation of \( \text{Na}^+ \text{-K}^+ \text{-ATPase} ,\(^{43,44}\) and to reduction in renal vascular resistance, particularly preglomerular arteriolar resistance vessels.\(^{15}\)

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**TABLE 1. PCR Primer Sets for Markers and SGC Subunits**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primers, 5’−3’</th>
<th>Genebank Accession No.</th>
<th>Expected Size, bp</th>
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<tbody>
<tr>
<td>CAMK</td>
<td>CTCCGACAGAAAGACTCGCAA</td>
<td>J05072</td>
<td>255</td>
</tr>
<tr>
<td></td>
<td>CAGGATCACCCAGGGACTGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2N35</td>
<td>GTGCTCATGACAGGCTGTGCA</td>
<td>M98802</td>
<td>213</td>
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<tr>
<td></td>
<td>CCTAACAACTGTGAAATCCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAK</td>
<td>ACGACGATGAGCAGATCC</td>
<td>X53234</td>
<td>187</td>
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<tr>
<td></td>
<td>TACTGGTCATTGCCAACCTAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCA</td>
<td>CCGCGCTGTTGTCATTGTCGG</td>
<td>J05767</td>
<td>286</td>
</tr>
<tr>
<td></td>
<td>ATCAATCTGCGAAAGGACAGC</td>
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<td></td>
</tr>
<tr>
<td>ET-2</td>
<td>AGCTGCCAGACTGACGTG</td>
<td>U02091</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>GCTCGGACCAAAGGATTTGGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGC ( \alpha_1 )</td>
<td>TGGGGAAGCTGAAAGCCAAC</td>
<td>M36075</td>
<td>438</td>
</tr>
<tr>
<td></td>
<td>TCACATGTGGATAGGTTCTTGG</td>
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<tr>
<td>SGC ( \beta_1 )</td>
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<td>M22562</td>
<td>284</td>
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<tr>
<td></td>
<td>GATTTTGCGGAAACGACAGCAC</td>
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<td>SGC ( \beta_2 )</td>
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<tr>
<td></td>
<td>TCACGTGGCCCATATTCTCCAC</td>
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</tbody>
</table>

PCR primers used in the analysis of rat-hamster radiation hybrid panels for loci on rat chromosomes 2 and 5. Primers are based on Genebank sequences.\(^{15,16}\)
A decrease in the sensitivity of guanylyl cyclase to NO has been reported in the SS/Jr kidney and postulated to play a central role in the pathogenesis of salt sensitivity. We have recently reported that mRNA and protein for the β2 subunit of guanylyl cyclase are increased and for the β1 subunit are decreased in the kidney of Dahl salt-sensitive versus salt-resistant rats. This suggests a molecular basis for decreased renal guanylyl cyclase activity in the Dahl salt-sensitive rat, i.e., an increase in the less NO-sensitive α1/β2 versus the more active α1/β1 form. However, a critical understanding of guanylyl cyclase subunit isoforms in renal salt handling requires localization and specific quantification of subunits within the kidney to determine heterodimer formation and the import of changes in subunit expression in vivo.

Cosegregation of SGC gene loci does not prove that guanylyl cyclase is the actual QTL for salt sensitivity. Nevertheless, the genetics combined with pharmacological and physiological data demonstrating (1) the link between NO signaling and blood pressure and salt sensitivity and (2) the presence of both reduced sensitivity to NO and NO-stimulated cGMP formation in the Dahl salt-sensitive rat make the hypothesis reasonable that a reduction in guanylyl cyclase activity contributes to or leads to salt sensitivity in the Dahl rat. Deng et al have shown that a blood pressure QTL is contained in the region spanning the D2N35 and NEP loci (which includes the NAK and CAMK loci) on chromosome 2 using congeneric strains. Further congeneric studies with greater resolution of the region will contribute to establishing the physiological link between SGC and salt sensitivity in the Dahl rat.

Studies of human populations have not reported an association between SGC and hypertension or salt sensitivity. The possibility that the genes for guanylyl cyclase are candidate genes in human salt sensitivity warrants their further investigation. If linkage to blood pressure is confirmed, they may serve as diagnostic or perhaps novel therapeutic targets.

Acknowledgments
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References
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